In the specification:

Please delete the paragraph beginning at page 12, line 17, and replace it with the following paragraph:

f1

Immunoassay profiles of fractions from SUPEROSE 12™ (Pharmacia) column chromatography of a pooled urine concentrate from pregnant women.

Please delete the paragraph beginning at page 76, line 12, and replace it with the following paragraph:

Characteristics of antibodies

sub G1

f2

A variety of hCG isoforms were employed to characterize the new antibodies described in this report and the nomenclature and characteristics of each of the reagents employed is summarized in Figure 11. The carbohydrate groups in these hCG isoforms as well as the percent nicking were analyzed in an earlier study (26) and are directly relevant for defining the nature of these new antibodies in this report.

Please delete the paragraph beginning at page 76, line 22, and replace it with the following paragraph:

sub G2

f3

Two antibodies designated B151 and B152 were selected by the use of radiolabeled hCG isoforms, chorioCG C5 and pregnancy hCG CR 127. Each displayed preferential binding to C5 as compared to CR 127 since this was the selection criterion. However, upon performing liquid phase immunoassays and calculating affinity constants, it was clear that these two antibodies were very different in specificity (Figure 12). It was found that

antibody B151 had one order of magnitude higher affinity both for C5, which is nicked and hyperglycosylated choriocarcinoma hCG, and for CR 127 hCGn (813) as compared to CR 127 hCG or nick-free CR 127(814) (see Figure 11 for reagent descriptions). B151 was clearly an antibody with a strong preference for binding to various forms of nicked hCG. Antibody B152 was different in that although it displayed one order of magnitude preference for C5 hCG over CR 127 hCG, it recognized the nicked and non-nicked forms of CR 127 hCG, hCG derived from normal pregnancies, to an equal extent.

Please delete the paragraph beginning at page 77, line 10, and replace it with the following paragraph:

Liquid Phase Assays

Figure 8 shows potency comparisons of liquid phase immunoassays of both B151 and B152 antibodies comparing competitors: 1. standard CR 127 pregnancy hCG (which has a 20% content of nicked hCG); 2. C5 chorio CG (100% nicked and hyperglycosylated); 3. 813, nicked CG made from CR 127 by purification, and 4. 814, non-nicked hCG derived from CR 127. The labeled ligand was C5 chorio CG. It is apparent that B151 (Fig 8A) shows a preference for nicked forms of hCG. C5 chorio-CG or 813 hCGn bind with similar affinities. The slightly lower potency of 813 hCGn may be ascribed to its 20% contamination with non-nicked hCG. B152 only shows a preference to C5, the hyperglycosylated chorio CG (Fig 8B). 813 hCGn is no more potent a competitor than nick-free 814 hCG.

Please delete the paragraph beginning at page 77, line 26, and replace it with the following paragraph:

Immunometric Two Site Assays

A variety of two site antibody formats were tested. Figure 13 displays these results. It is apparent that B151 cannot bind simultaneously with antibodies (designated by us as site IV) (27) to the beta subunit and beta subunit core (B201 and B204) nor with antibodies directed towards the determinant which exists in heterodimeric hCG as represented by antibody B109 (site III, to which A109 also belongs) (27). In contrast, a general beta antibody which binds to the most common and potent hCG antigenic site previously designated by us as site II (B108 or B207) binds well simultaneously with both B151 and B152 antibodies. B152 binds simultaneously to all antibodies tested except for those to the beta COOH-terminal region (CTP) (28) in contrast to B151 which binds well to CTP antibodies. B151 may represent a newly revealed hCG epitope which only exists on nicked hCG as reported in this manuscript.

Please delete the paragraph beginning on page 79, line 1, and replace it with the following paragraph:

Characteristics of the B152-B207-I^{125} radioimmunometric two-site assay

In order to better understand what this assay is measuring, we compared the relative binding potencies of a series of isoforms of hCG shown in Figure 9 (also see methods): 1. C5, choriocarcinoma hCG 2. 814, non-nicked hCG. 3. 813, nicked hCG (80% nicked). 4. M4 mole-

derived hCG, 98% nicked hCG with negligible hyperglycosylation. 5. MIA hCG, non-nicked and not significantly hyperglycosylated but missing 80% of the hCG beta COOH-terminus. The B152 two-site assay prefers to bind to C5, its immunogen, but shows nearly equal recognition of both 813 and 814, nicked and non-nicked hCG of normal pregnancy. This confirms that B152 does not display significant preference for the nicked form of hCG but rather for the form with carbohydrate differences. This is also confirmed by the potency of M4 which is also 100% nicked as is C5 but is not hyperglycosylated and displays a potency similar to CR 127 hCG whether nicked or non-nicked. MIA is the least potent ligand and is the only one missing most of its beta COOH-terminal peptide confirming the role of this region in the B152 epitope.

Please delete the paragraph beginning on page 79, line 24, and replace it with the following paragraph:

In order to further explore the nature of the B152 binding site, a commercially available peroxidase-labeled general hCGβ antibody (4001) was employed as a detection antibody in a two-site enzyme immunometric system. Eight different hCG forms were evaluated in this system illustrated in Figure 10. Results are analyzed in terms of relative immunopotency (based on the slope of the regression line) in Figure 14. Linear regression correlation analysis was performed to compare the relationship of the immunopotencies of preparations 814, C5, M4, C7 and P8 one at a time with the carbohydrate differences (Figure 11) as well as nicking differences among the heterodimeric isoforms of hCG. The correlation results for each comparison are as